CLAIMS

What is claimed is:

1. A method for determining an unknown starting quantity of
    a target nucleic acid sequence in a test sample, the
    method comprising the steps of:
    a) amplifying the unknown starting quantity of the
        target nucleic acid sequence in the test sample;

    b) amplifying a plurality of known starting quantities
        of a calibration nucleic acid sequence in respective
        calibration samples;

    c) determining a respective threshold value for each of
        the known starting quantities of the calibration
        nucleic acid sequence in the calibration samples and
        for the target nucleic acid sequence in the test
        sample, wherein the threshold value is determined
        for each nucleic acid sequence in a respective
        sample by:

        i)   measuring, at a plurality of different times
             during amplification, at least one signal whose
             intensity is related to the quantity of the
             nucleic acid sequence being amplified in the
             sample;

        ii) deriving a growth curve from the measurements
             of the signal;

        iii) calculating a derivative of the growth curve;

        iv) identifying a characteristic of the derivative;
             and

v) determining a threshold value associated with the characteristic of the derivative;

d) deriving a calibration curve from the threshold values determined for the known starting quantities of the nucleic acid sequence in the calibration samples; and

e) determining the starting quantity of the target nucleic acid sequence in the test sample using the calibration curve and the threshold value determined for the target sequence.

2. The method of claim 1, wherein each of the threshold values comprises a cycle number.

3. The method of claim 1, wherein each of the threshold values comprises an elapsed time of amplification.

4. The method of claim 1, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a positive peak of the second derivative.

5. The method of claim 1, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the

characteristic comprises a negative peak of the second derivative.

6.   The method of claim 1, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a zero crossing of the second derivative.

7.   The method of claim 1, wherein the step of calculating a derivative of the growth curve comprises calculating a first derivative of the growth curve, and wherein the characteristic comprises a positive peak of the first derivative.

8.   The method of claim 1, wherein the step of calculating a derivative of the growth curve comprises calculating second derivative values of the growth curve at a number of different measurement points in the reaction to yield a plurality of second derivative data points, the characteristic comprises a positive peak of the second derivative, and the step of determining the threshold value associated with the positive peak comprises:

    i)   fitting a second order curve to the second derivative data points; and

    ii)   calculating the threshold value as the location of a peak of the second order curve.

9. A method for determining an unknown starting quantity of a target nucleic acid sequence in a test sample, the method comprising the steps of:

   a) amplifying the target nucleic acid sequence and a first internal control in a first nucleic acid amplification reaction, wherein the first internal control comprises a second nucleic acid sequence different than the target nucleic acid sequence in the test sample;

   b) amplifying a first standard and a second internal control in a second nucleic acid amplification reaction, wherein the first standard comprises a first known starting quantity of a calibration nucleic acid sequence different than the second nucleic acid sequence, and wherein the second internal control comprises the second nucleic acid sequence;

   c) amplifying a second standard and a third internal control in a third nucleic acid amplification reaction, wherein the second standard comprises a second known starting quantity of the calibration nucleic acid sequence, the third internal control comprises the second nucleic acid sequence, and the starting quantity of the second nucleic acid sequence is substantially equal in each of the amplification reactions;

   d) determining a respective threshold value for each of the standards, each of the internal controls, and the target nucleic acid sequence in the test sample;

   e) normalizing the threshold value determined for the target nucleic acid sequence in the test sample to

the threshold value determined for the first
internal control;

f)   normalizing the threshold values determined for the
     first and second standards to the threshold values
     determined for the second and third internal
     controls, respectively;

g)   deriving a calibration curve from the known starting
     quantities and the normalized threshold values of
     the first and second standards; and

h)   determining the starting quantity of the target
     nucleic acid sequence in the test sample using the
     calibration curve and the normalized threshold value
     determined for the target sequence.

10. The method of claim 9, wherein each of the threshold
     values comprises a cycle number.

11. The method of claim 9, wherein each of the threshold
     values comprises an elapsed time of amplification.

12. The method of claim 9, wherein a respective threshold
     value is determined for each nucleic acid sequence by:

     i)   measuring, at a plurality of different times during
          amplification of the nucleic acid sequence, at
          least one signal whose intensity is related to the
          quantity of the nucleic acid sequence being
          amplified;

ii) deriving a growth curve from the measurements of the signal;

iii) calculating a derivative of the growth curve;

iv) identifying a characteristic of the derivative; and

5   v) determining the threshold value associated with the characteristic of the derivative.

13. The method of claim 12, wherein the step of calculating a derivative of the growth curve comprises calculating a

10   second derivative of the growth curve, and wherein the characteristic comprises a positive peak of the second derivative.

14. The method of claim 12, wherein the step of calculating a derivative of the growth curve comprises calculating a

15   second derivative of the growth curve, and wherein the characteristic comprises a negative peak of the second derivative.

20 15. The method of claim 12, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a zero crossing of the second derivative.

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16. The method of claim 12, wherein the step of calculating a derivative of the growth curve comprises calculating a

first derivative of the growth curve, and wherein the characteristic comprises a positive peak of the first derivative.

5 17. The method of claim 12, wherein the step of calculating a derivative of the growth curve comprises calculating second derivative values of the growth curve at a number of different measurement points in the reaction to yield a plurality of second derivative data points, the

10   characteristic comprises a positive peak of the second derivative, and the step of determining the threshold value associated with the positive peak comprises:

   i)   fitting a second order curve to the second derivative data points; and

15 ii) calculating the threshold value as the location of a peak of the second order curve.

18. The method of claim 9, wherein the step of normalizing the threshold value determined for the target nucleic

20   acid sequence in the test sample to the threshold value determined for the first internal control comprises dividing the threshold value determined for the target nucleic acid sequence by the threshold value determined for the first internal control.

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19. The method of claim 9, wherein the step of normalizing the threshold values determined for the first and second standards to the threshold values determined for the

second and third internal controls, respectively,
comprises dividing the threshold values determined for
the first and second standards by the threshold values
determined for the second and third internal controls,
5   respectively.

20. A method for determining an unknown starting quantity of
    a first nucleic acid sequence in a test sample, the
    method comprising the steps of:

10   a) amplifying in one reaction vessel the first nucleic
         acid sequence, a first standard, and a second
         standard, wherein the first standard comprises a
         known starting quantity of a second nucleic acid
         sequence different than the first nucleic acid
15   sequence, and wherein the second standard comprises
         a known starting quantity of a third nucleic acid
         sequence different than the first and second
         sequences;

     b) determining a respective threshold value for the
20   first standard, the second standard, and the first
         nucleic acid sequence;

     c) deriving a calibration curve from the known starting
         quantities and from the threshold values determined
         for the first and second standards; and

25   d) determining the starting quantity of the first
         nucleic acid sequence using the calibration curve
         and the threshold value determined for the first
         nucleic acid sequence.

21. The method of claim 20, wherein each of the threshold values comprises a cycle number.

22. The method of claim 20, wherein each of the threshold values comprises an elapsed time of amplification.

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23. The method of claim 20, wherein a respective threshold value is determined for each nucleic acid sequence by:

    i)   measuring, at a plurality of different times during amplification of the nucleic acid sequence, at least one signal whose intensity is related to the quantity of the nucleic acid sequence in the vessel;

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    ii)   deriving a growth curve from the measurements of the signal;

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    iii) calculating a derivative of the growth curve;

    iv) identifying a characteristic of the derivative; and

    v)   determining the threshold value associated with the characteristic of the derivative.

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24. The method of claim 23, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a positive peak of the second derivative.

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25. The method of claim 23, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a negative peak of the second derivative.

26. The method of claim 23, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a zero crossing of the second derivative.

27. The method of claim 23, wherein the step of calculating a derivative of the growth curve comprises calculating a first derivative of the growth curve, and wherein the characteristic comprises a positive peak of the first derivative.

28. The method of claim 23, wherein the step of calculating a derivative of the growth curve comprises calculating second derivative values of the growth curve at a number of different measurement points in the reaction to yield a plurality of second derivative data points, the characteristic comprises a positive peak of the second derivative, and the step of determining the threshold value associated with the positive peak comprises:

    i)   fitting a second order curve to the second derivative data points; and

ii) calculating the threshold value as the location of
a peak of the second order curve.